Title: Spatial bias in dietary studies can limit our understanding of the feeding ecology of large carnivores

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Abstract

Many large carnivores have broad geographical ranges, encompassing ecosystems with a different prey base. Our understanding of their diet could therefore be biased by the spatial concentration of dietary studies into few areas. We propose a protocol to divide the geographical range of large carnivores, into areas that are homogeneous with respect to available food sources, by using the grey wolf (*Canis lupus*) in Italy, as a case study.

We mapped the potential maximum distribution of wolves, on a 10 km grid (n = 2,497), and then performed cluster analysis to classify cells according to their: *i*) abundance of domestic and wild ungulates, *ii*) suitability for the coypu (*Myocastor coypus*) and *iii*) landscape anthropization.

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Finally, we checked the percentage of cells in each cluster that were covered by dietary studies in 2007-2013, 2014-2018 and 2019-2023.

The distribution range of wolves in Italy can be divided into 5 areas, characterized by different food sources but also by a different spatial coverage from dietary studies. The Alps and some sectors of the Apennines, with low anthropization and abundant wild ungulates, were oversampled. More anthropized areas in Central and Southern Italy, rich in sheep and wild ungulates, as well as anthropized lowlands, with abundant food waste and coypu, were undersampled. Finally, no study was carried out in intensive farming districts of Northern Italy.

Our protocol indicates that future studies about the diet of wolves in Italy should focus on anthropized landscapes. There, the consumption of pets could trigger wolf persecution and pathogen transmission, and predation on coypu and the consumption of food waste could increase the exposure to toxic compounds.

More broadly, our protocol can improve our understanding about the feeding ecology of large carnivores, as it can be used to: *i*) assess and put into perspective meta-analytic findings, *ii*) identify knowledge gaps arising from spatial bias and prioritize new studies in undersampled areas and *iii*) design sampling schemes for large-scale research.

Keywords: mammals; diet; predation; carnivores; synthesis research

Introduction

Meta-analyses summarize and advance existing knowledge about the biology, ecology, evolution, and conservation of animals and plants (Gurevitch et al., 2018), and reveal the occurrence, magnitude, and spatiotemporal variation of ecological dynamics (Peters, 2010).

However, the increase in meta-analyses in ecology and evolution, since the early 2000s (Cadotte et al., 2012; Vetter et al., 2013), came hand-in-hand with the growing awareness that aggregating different studies is challenging, particularly in fast-evolving fields like ecology, ethology or evolutionary biology. On the one hand, the reliability of a meta-analysis depends upon the transparency and standardization of data collection protocols across studies, with differences in measurements and/or analytical methods resulting into spurious findings (Gurevitch et al., 2018; Nakagawa et al., 2017; Whittaker, 2010). On the other hand, the generalizability of its conclusions depends upon the quality of the sampling strategy and, for ecological dynamics that vary in space, the geographical balance of the various studies that are summarized. A well-known example is spatial-bias, with most peer-reviewed studies being conducted in the Global North, in more accessible areas, or in parks and natural reserves (Christie et al., 2020; Di Marco et al., 2017; Hughes et al., 2021).

Large carnivores have been the focus of many reviews and meta-analyses that covered, among the others, their long-term population dynamics and range shifts (Ingeman et al., 2022; Jacobson et al., 2016; Murphy et al., 2022; Strampelli et al., 2022; Wolf and Ripple, 2017), movement ecology (Gonzalez-Borrajo et al., 2016; Morales-González et al., 2022), genetic diversity (Hindrikson et al., 2017; O’Brien et al., 2017), and interspecific relationships (Franchini and de las Mercedes Guerisoli, 2023; Périquet et al., 2014). Perhaps, one of the most covered topics is carnivores diet (Table 1), due to its major implications for ecosystem dynamics. Many large carnivores rely on wild ungulates as a prey base: once these are depleted, they can either perish (Carbone et al., 2011; Wolf and Ripple, 2017) or shift to alternative food sources (Creel et al., 2017), including livestock or human food waste, two food sources that may create conflicts with humans (van Eeden et al., 2017; Newsome et al., 2014). Reviews about the diet of large carnivores are therefore fundamental to understand how they can respond to human impacts on wildlife and ecosystem, and therefore result crucial to plan their management. It may be the case of top predators with a wide dietary breadth, which, differently from strictly specialist carnivores, can shift to livestock or other anthropogenic resources according to the local availability of the different items (Ferretti et al., 2020), which in turn depends on human impacts and landscape transformations (Kuijper et al., 2024; Newsome et al., 2014).

Understanding dietary breadth was the scope of many reviews about large carnivores, particularly for adaptable species with a large geographical range and living alongside humans. Nevertheless, spatial bias is likely to limit the generalizability of their findings. As resource selection is an adaptive process (Manly et al., 2007), with predators adapting to available prey in a certain area, a review based on studies covering only part of the geographical range and only specific environmental types among those inhabited by a large carnivore is unlikely to capture its whole dietary breadth. This problem is exacerbated for those species whose geographical distribution expands or shifts through time, often to environmental types which were not included in their former range. Some large carnivores in Europe and North America are in facts recovering part of their historical range (Chapron et al., 2014; Miller et al., 2013), with human-dominated landscapes being increasingly represented within their distribution ranges (Kuijper et al., 2024, Zanni et al., 2023). As dietary studies require time for data collection and processing, the rhythm to which they are published might not match these fast spatiotemporal dynamics, resulting in increased spatial bias through time.

While spatial-bias has already been mentioned as a potential limitation for research about large carnivore diet (Newsome et al. 2016), to the best of our knowledge no study quantified it, nor proposed a workflow to detect it. Evaluating if published literature is biased, with respect to the ecological conditions characterizing the whole range of a certain species, can be used both beforehand and retrospectively. By checking for spatial bias in advance, researchers can decide whether existing literature is suitable to carry out a meta-analysis or even to study the diet of a certain species in a geographical region where no study has been conducted before. Conversely, the post-hoc exploration of spatial-bias could be used to put existing scientific evidence into perspective.

In this study we aim to show how spatial and ecological bias in dietary studies involving large carnivores could be assessed and evaluated, by using the expansion of the gray wolf (*Canis lupus*) in Italy as a case study. Gray wolf can indeed be considered the most successful large carnivore at recolonizing the human-dominated portion of its former range in Europe (Kuijper et al. 2024) and Italy is among the European countries that, since the 1970s, had the most marked increase in wolves (Boitani et al., 2022, Zanni et al. 2023). Starting from a small population of a few hundred individuals in remote areas of Central Italy (Zimen and Boitani, 1975; Cagnolaro et al., 1974), at least 2,945 – 3,608 individuals are nowadays thought to be present (La Morgia et al., 2022), upsetting the scenarios for wolf conservation and coexistence in the country.

Even though wolves are known to have a wide trophic niche, and can include in their diet also unexpected resources (Adams et al., 2010; Barocas et al., 2018; Mohammadi et al., 2019; Roffler et al., 2022) most reviews regard wild ungulates, or alternatively livestock, as the cornerstone of their diet, with other food sources playing a minor role (Capitani et al. 2004; Janeiro-Otero et al., 2020; Meriggi et al., 2011; Mattioli et al. 2011, Meriggi and Lovari, 1996; Mori et al., 2017; Newsome et al., 2016; Zlatanova et al., 2014). However, if wolves had truly relied on wild ungulates, it is unclear how they could be colonizing peri-urban areas and croplands (Torretta et al., 2022; Zanni et al., 2023), where ungulates are less abundant and where recent evidence suggests they rely on alternative food sources (Ciucci et al., 2020; Ferretti et al., 2019; Musto et al., 2024).

The surprising speed of wolf expansion in Italy, leading to the recovery of most of its historical range (differently from other European countries, e.g., Spain, Clavero et al., 2023), made Italy a perfect workbench to highlight potential spatial biases in wolf diet literature. We did so by considering three temporal windows for which wolf occupancy data are available (2007-2012, 2013-2018 and 2019-2023) and then by comparing the ecological conditions characterizing *i*) the spatial distribution of wolves in Italy and *ii*) that of study areas of wolf diet research.

Methods

Collection of studies and inclusion criteria

Data collection adopted a threefold approach. First, we extracted all those studies that were mentioned in reviews about wolf diet (Janeiro-Otero et al., 2020; Meriggi et al., 2011; Mori et al., 2017; Newsome et al., 2016; Zlatanova et al., 2014), and that were conducted in Italy between 2007 and 2023.

Moreover, we also searched for the keywords "*Canis lupus*", "*wolf*" and "*diet*" (similarly to Newsome et al., 2016) on three large datasets of scientific publications: Scopus, Web of Science, and Google Scholar. Then, from this second pool of studies, we selected those which had been carried out in Italy, and were published between 2007 and 2023. As the period when data had been collected was not always reported, and results were often not splitted between different years, we used the year of publication to assign each study to one of our three periods. By doing so we obtained insights about studies that were carried out after the most recent review about wolf diet (Janeiro-Otero et al., 2020), or that had been discarded by previous reviews, but whose spatial location was informative about potential spatial biases.

Finally, we also collected available gray literature about wolf diet in Italy. This included non-peer reviewed documents, such as dissertations of MSc and PhD students that had not been subsequently published in a peer-reviewed journal, or reports published by local authorities and protected areas.

As dissertations are not always adequately indexed on the archives of Italian universities, we used snowballing. First, we queried "*Dieta lupo*", the Italian translation of "*Wolf diet*" on university archives and Google. Then, starting from an initial sample of Msc thesis that we previously knew about, we asked mentors if they had supervised other students on the same topic, between 2007 and 2023. Finally, we also asked colleagues from other research groups if they could indicate some gray literature on the topic, until no new studies were detected. From the pool of studies that we had obtained, we retained those for which it was possible to understand where data had been collected, with respect to the grid used by the Ministry for the Environment to quantify wolf occupancy in 2019-2021 (La Morgia et al., 2022).

Although different methods for investigating wolf diet may provide different results (Klare et al., 2011), we did not discard studies according to the method they used as our meta-analysis focused on assessing spatial bias. So, we pooled together studies relying on scat analysis, barcoding, stomach contents and isotopes.

Quantification of spatial location, measurements and statistical analysis

We used a 10-km resolution grid produced by the Ministry of the Environment (La Morgia et al. 2022), to identify: *i*) the distribution of wolves, *ii*) environmental conditions and *iii*) the spatial coverage of studies about wolf diet in peninsular Italy.

For the 2019 – 2023 period, for peninsular Italy we used the whole grid produced by La Morgia et al. (2022), whose cells had an occupancy probability different from zero. Moreover, we also added those cells in the Alps, where the presence of wolves was confirmed (La Morgia et al., 2022). For the 2007-2013 and 2014-2018 periods, we used official distribution maps from official reports of the Habitat directive (http://reportingdirettivahabitat.isprambiente.it/). To ensure consistency between the three different periods these maps were aligned to the grid developed by La Morgia et al. (2022). A complete overview of wolf distribution in these three periods is available in Fig. 1.

Then, for each cell of the grid we extracted variables related to the main food sources available to wolves. These included: anthropization and the presence of domestic livestock, wild ungulates, and the coypu (*Myocastor coypus*).

Anthropization is an important factor affecting wolf diet, mostly through food waste, which is a nearly unlimited food source. Although wolves do not fully exploit carbohydrates (Axelsson et al., 2013), evidence from non-European countries indicate that they exploit food waste whenever available (Barocas et al., 2018; Mohammadi et al., 2019), probably by selecting for meat scraps and bones. Moreover, wolves living around human settlements could also prey on pets (Bassi et al., 2017; Kojola et al., 2022; Nowak et al., 2011). We quantified anthropization by calculating evenness of human density, quantified at a 1km resolution through the Global Human Settlement Layer (https://human-settlement.emergency.copernicus.eu/index.php), for each cell of our grid. Evenness was quantified through the Gini index, which varies from 0, when all the units of a sample have the same value of a certain measure, to 1 when one unit has the entire amount of that value. Therefore, the Gini index in our case was negatively associated with human presence, with cells having the lowest values being characterized by widespread human settlements and having a higher amount of food waste.

We also considered the abundance of domestic livestock, particularly sheep, cattle, and domestic pigs, that can be regularly preyed on by wolves (Gervasi et al., 2021). Moreover, the abundance of livestock could also account for the availability of carrion and slaughterhouses in the environment, important supplementary food sources for wolves (Ciucci et al., 2020; Ćirović and Penezić, 2019). For domestic livestock, we used 10km abundance projections generated by the Food and Agriculture Organization and structured in the Gridded Livestock of the World database (GLW4, https://data.apps.fao.org/catalog/dataset/15f8c56c-5499-45d5-bd89-59ef6c026704), related to the abundance of sheep, cattle, and domestic pigs.

For wild ungulates, we considered the five most preyed on by wolves in Italy: roe deer (*Capreolus capreolus*), deer (*Cervus elaphus*), wild boar (*Sus scrofa*), fallow deer (*Dama dama*) and Northern chamois (*Rupicapra rupicapra*) (Gazzola et al. 2007, Mattioli et al. 2011). We did not consider the mouflon (*Ovis gmelini musimon*), which is distributed with scattered populations in the Italian peninsula, although occasionally it could represent an important prey (Capitani et al. 2004). Moreover, we did not consider the Sika deer (*Cervus nippon*), because its distribution in Central and Northern Italy is still uncertain (Mori et al., 2024). Neither we considered the Alpine ibex (*Capra ibex*), as it seems to play a minor role in the diet of alpine wolves (Palmegiani et al. 2013). For wild ungulates, we relied on 10-km hunting yield density maps elaborated within the ENETWILD project (ENETWILD consortium, 2022), using them as relative indexes for the local abundance of each wild ungulate species. Although the ENETWILD maps predict low values for the abundance of a certain species, even in areas lying outside of its actual distribution range, we used them as they were the only available information about the occurrence and abundance of multiple ungulate species at the national scale, in Italy. Moreover, in cluster analysis (see the following lines), areas with a different prey base were identified mostly by high densities of the various species, and therefore this bias was not deemed to affect our results.

Finally, we also included the potential environmental suitability of the Italian peninsula for the coypu. Recent studies found out that the coypu can be an important prey, in some agricultural ecosystems of Central and Northern Italy (Musto et al., 2024; Ferretti et al., 2019), probably because it is easy to prey and can attain very high densities, providing wolves with a relevant biomass (Balestrieri et al., 2016). As no abundance map was available for this species, we rather use the potential suitability of the Italian landscape, at a 1 km resolution, obtained from Schertler et al. (2020). For each cell of our grid, we calculated the median for the abundance of wild ungulates and livestock and the geometric mean for the suitability for the coypus. Although wolves in many areas of Central and Northern Europe regularly prey on other aquatic rodents, such as the Eurasian beaver

(*Castor fiber*, Gable et al., 2018), we did not include this species, because its population in Central and Northern Italy is still extremely small and confined to few areas (Bertolino et al., 2024).

Finally, we assigned our studies to the various cells of the grid. As each study provided us with different information about its study area, and the location where data had been collected, we identified the location of each study area as the geographical center of the area where biological samples had been collected. Then, we assumed that wolves for which biological samples had been collected on the centroid, could have moved in a home range of approximately 113 km^2 (Mancinelli et al., 2018; Mattioli et al., 2018) and so we generated a buffer with a radius of 6 km around each point and classified all those cells of the grid that overlapped with it. As our scope was to assess the spatial coverage of existing studies about wolf diet, and because many studies refer to the same research project, we only classified the cells of our grid as being covered by studies or not, with a dichotomous variable.

Finally, we identified environmentally homogeneous areas within the entire Italian peninsula, based on: *i*) the Gini index of human density, *ii*) the median abundance of roe deer, red deer, wild boar, fallow deer and Northern chamois, *iii*) the median abundance of domestic pigs, sheep and cattle, i*v*) the geometric mean of the potential suitability for the coypu. To this end, we used the CLARA algorithm, an extension of Partitioning Around Medoids cluster analysis (Kaufman and Rousseeuw, 2009). We chose the CLARA algorithm due to the high number of cells (n = 2,497) and its robustness against non-normal data and outliers. The number of clusters was chosen by graphically exploring the silhouette width method, the elbow method, and the gap statistics method (Kassambara, 2017). Before clustering our cells, we standardized and centered our variables.

Once we identified environmental clusters, we graphically inspected how studies about wolf diet were distributed between different clusters, across the three different periods. Although we clusterized environmental variables in the entire Italian peninsula, we then only explored coverage in those cells that corresponded to the maximum distribution range of the wolf. This range (n. cells

= 1,974) was obtained by considering all cells where the species was reported at least in one of the three time periods. We calculated both *i*) the portion of dietary studies being conducted in each different cluster and *ii*) the portion of cells of each cluster being involved in at least a dietary study, both overall and across the three different time periods.

Statistical analyses were carried out in R (R Core Team 2023). A completely reproducible dataset and software code are available at https://osf.io/76cx4/

Results

Our final dataset included 36 studies: 27 of them were published in peer-reviewed journals, 8 of them were MSc dissertations and 1 was a study published in the proceedings of a scientific conference (see the file "StudiesDiet_20240624.xlsx" in the "Data" folder of Supplementary Information). Most studies were published during the 2019-2023 (n = 15) and the 2014-2018 periods (n = 13). As for MSc dissertations, 6 studies out of 8 were from the 2019-2023 period, probably because older dissertations had not been archived in a digital format.

Cluster analysis identified 5 groups of areas that were homogeneous in terms of food resources (Fig. 2-4). The first group (Cluster 1) coincided with the Alps and with high-elevation areas of Central Apennines. Cells in this cluster had a high abundance of wild ungulates, particularly of Northern chamois and red deer, extremely low anthropization and little animal husbandry. These areas were unsuitable for the coypu.

The second group (Cluster 2) included areas with sheep herding, medium-low values of anthropization, high abundance of roe deer, wild boar, and fallow deer, and that were moderately suitable for the coypu. These areas have low abundance of the red deer. The third group (Cluster 3) included cells with high abundance of wild ungulates, including the red deer, but characterized by lower values of anthropization, sheep herding and suitability for the coypu than cells from Cluster

2. Overall, Cluster 2 and Cluster 3 were common in Central and Southern Italy, where they account for most Apennines areas, and in the Prealps in Northern Italy.

The fourth (Cluster 4) and the fifth group (Cluster 5) included anthropized areas. Namely, Cluster 4 included cells with high urban sprawl and the highest suitability for the coypu, characterized by little animal husbandry and intermediate abundances of the roe deer and the wild boar. Cluster 5 instead corresponded to areas of intensive animal husbandry, with high densities of sheep, cattle and domestic pigs. Cluster 5 was also suitable for the coypu and had intermediate values of anthropization. Overall, all cells from Cluster 5 and most cells from Cluster 4 occurred in the Po Plain in Northern Italy, but some cells from Cluster 4 also occurred in lowlands of Central and Southern Italy.

When considering the entire 2007-2023 period, most cells covered by studies about wolf diet were in Cluster 3 (43%). The proportion was lower in Cluster 1 (28%), Cluster 2 (22%) and Cluster 4 (8%). Conversely, when checking the percentage of cells that covered by studies in each cluster, both Cluster 1 and Cluster 3 attained the highest coverage, with 19% and 18% of cells. Coverage was much lower for Cluster 4 and Cluster 2 (5%). Moreover, although Cluster 5 accounted only for an area of 400km2 in the distribution range of wolves, its cells were never interested by dietary studies.

When considering the spatial distribution of dietary studies through time, we noticed that the distribution of cells interested by studies has become more even between 2007-2013 and 2019-2023, with the progressive inclusion of Cluster 4 (Table 2).

Discussion

Systematic reviews are crucial to summarize existing knowledge about the feeding ecology of species of conservation concern, and prone to conflict with humans, such as large carnivores. However, spatial bias can limit the generalizability of their findings. In this study we showed how researchers can quantify the magnitude of spatial bias, by identifying areas that result homogeneous in terms of prey they can offer to a large carnivore and then checking the allocation of existing studies between them. Namely, we used data about the distribution of important food sources for the gray wolf in Italy, to identify 5 ecologically homogeneous areas at the national level, and then understand the extent to which these were interested by dietary studies that had been carried out since 2007.

From a research viewpoint, our protocol can be used either before conducting a systematic review or after having produced one. In the first case, whenever existing literature is limited to specific environments, researchers might decide not to synthesize knowledge at all, as knowledge gaps could interest a significant portion of a species range. Alternatively, and perhaps more pragmatically, researchers could review existing literature, and then put their findings into perspective, by specifying that areas with certain types of prey species or other source of food were not covered. Identifying spatial bias in existing dietary studies could also be useful to plan future research, by assisting the design of large-scale surveys for scat collection. To ensure unbiased findings, or at least minimize bias associated with scat collection, Steenweg et al., (2015) suggested the adoption of spatially balanced random sampling, like generalized random tessellation stratification. Although spatially balanced sampling is robust against unobserved bias (Kermorvant et al., 2019), we believe that our approach, by identifying strata that are homogeneous in terms of environmental resources, could be useful to develop advanced sampling designs with a higher accuracy (Robertson and Price, 2024).

There is much to be gained from a similar process both in terms of ecological research and management, and we will make a few examples from our case study about wolves.

Concerning the 5 homogeneous areas we identified by clustering food sources; our findings clearly highlight that existing literature about the diet of wolves is severely unbalanced. Most research referred to Cluster 3 and Cluster 1, areas with little landscape anthropization and abundant wild ungulates. In these areas wolves have been found to rely mostly on deer and wild boar, which are nevertheless much less abundant in Cluster 4 and 5. However, with the progressive recolonization of the Italian peninsula by wolf packs (Bassi et al., 2015), wolves indeed expanded in human-modified areas as those included in Clusters 4 and 5 (Torretta et al., 2022; Zanni et al., 2023). Since these areas are also those more likely to host conflicts with humans, due to their high human presence and activities, the knowledge gaps concerning wolf ecology in these areas may prevent the application of evidence-based conservation and conflict management policies (Kuijper et al., 2024). It indeed remains unclear the extent to which wolves in these human-dominated areas could shift to coypus, food waste, livestock, pets and animal byproducts, with major possible alterations of their fitness and behavior. Indeed, beside the direct effects on behavior, diet shifts towards anthropogenic items may also induce modifications of wolf's genetics through an increased proximity with commensal domestic or feral dogs, likely more abundant in human-dominated areas, ultimately leading to an enhanced likelihood of hybridization (Hughes & Macdonald, 2013). Moreover, the analysis of scats collected in these environments, coupled with a genetic assessment of the hybridization level, can also be useful to see if wolves and wolf-dog hybrids segregate their trophic niche, something that does not seem to happen in less anthropized environments (Bassi et al., 2017). In the case of peri-urban wolves, the presence of remains attributable to pets was observed in the stomach contents (see Appendix 1). The consumption of domestic cats can also be inferred from the detection in the intestinal matrix of wolves of viruses typical of felines (e.g., Feline Panleukopenia Virus, Balboni et al., 2021). Beside directly threatening wolves through an increased share of parasites and other pathogens, and being their loss both an emotional and economic harm for owners, the predation of pets may drive negative attitudes towards wolf conservation (Lescureux & Linnell, 2014). Fulfilling our knowledge gaps on the wolf dietary patterns in anthropized environments, hosting more domestic dogs and cats, may thus enhance our understanding of wolf predation on pets and its possible implications for wolf conservation.

Also, researchers frequently attempt to monitor temporal changes in the diet of large carnivores. In our case study, if we want to monitor changes in the diet of wolves through time, apart from being sure about potential issues of sampling bias (Gable et al., 2015), it is important that comparisons are based on areas with similar prey composition. For example, our findings indicate that dietary studies in livestock districts (Cluster 5, currently not sampled) are urgently needed to quantify dietary habits of wolves inhabiting these environments. Moreover, assessing dietary shifts between 2007-2013 and 2019-2023 by pooling together multiple studies with a cross-sectional approach is questionable, as studies were initially concentrated in less anthropized environments. Combining longitudinal studies carried out on single wolf packs (Bassi et al. 2020) or at least populations (e.g. Mattioli et al., 1995; 2011), seems certainly more appropriate for this purpose.

Our protocol could also be used to study the role played by underrated prey in the diet of a large carnivore. For example, areas from Cluster 4 and Cluster 5 are also rich in coypu. So far, few studies explored the extent to which wolves could rely on this species (Ferretti et al., 2019) and none was carried out in areas, like Cluster 4 and 5, where domestic or wild ungulates are scarce. As coypu are easy to catch in agricultural channels and could attain significant densities (Balestrieri et al., 2016), it is plausible that they are indeed a major prey for wolves. Empirical evidence seems to confirm this point: by analyzing the stomach content of 64 wolves that were found dead in the Po Plain (Cremona, Mantua, and Bologna provinces), we found remains of coypu in 10 individuals (15.6%). Beyond the necropsy findings, we believe that rodents (rats and coypus) are increasingly contributing to the diet of peri-urban wolves in Italy, as 61.8% of the wolf carcasses analyzed by Musto et al. (2024) were positive to the presence of Second-Generation Anticoagulant Rodenticides (SGARs), particularly in highly anthropized areas, due to probable ingestion of poisoned rodents. Therefore, it is plausible that rodents, particularly coypu (similarly to beavers, Gable et al., 2018), might be an important species which is fueling the expansion of wolves in lowlands, particularly that of dispersing individuals or couples. This could explain the significant expansion of wolves along the Po Plain, where two packs are nowadays present in the Po delta.

In conclusion we provided a rigorous and standardized approach to assess spatial and ecological bias in dietary studies, which may be profitably replicated with other large carnivores and even other animal group such as scavengers, herbivores, marine predators and mesocarnivores to identify and address gaps in our knowledge of their feeding ecology, with the ultimate goals to increase our understanding of the context-dependent variations of their ecological impacts and to improve their conservation.

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Supplementary material

The supplementary information, as well as the reproducible data and software code, are available at: https://osf.io/76cx4/

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Ethical approval

Not applicable

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CRediT authorship contribution statement

Conceptualization: JC, RB, CM, EB **Methodology:** JC, RB, CM, EB **Software:** JC **Validation:** JC, RB, CM, EB, GV, AB, MD, MS, MA **Formal analysis:** JC **Investigation:** JC, RB, CM, EB, GV, AB **Resources:** GV, AB, MD, MS, MA **Data curation:** CM, EB, GV, AB **Writing - original draft:** JC, RB, CM, EB **Writing- review and editing:** JC, RB, CM, EB, GV, AB, MD, MS, MA **Visualization:** JC, RB, CM, EB **Supervision:** MD, MS, MA **Project administration:** MD, MA **Funding Acquisition:** GV, AB, MD, MS, MA

Figures

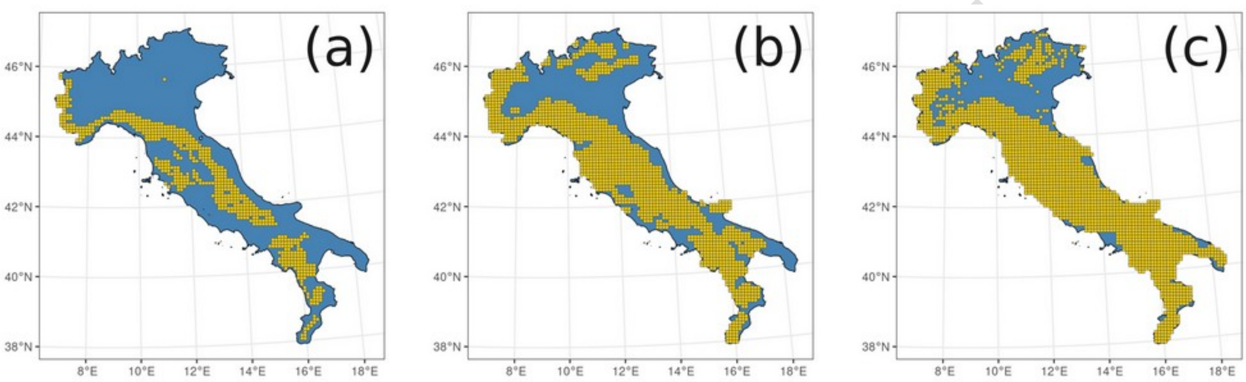

Fig. 1. Areas of the Italian peninsula where wolves were present in 2007-2013 (a), 2014-2019 (b) and 2019-2023 (c). For data sources please see the Methods section.

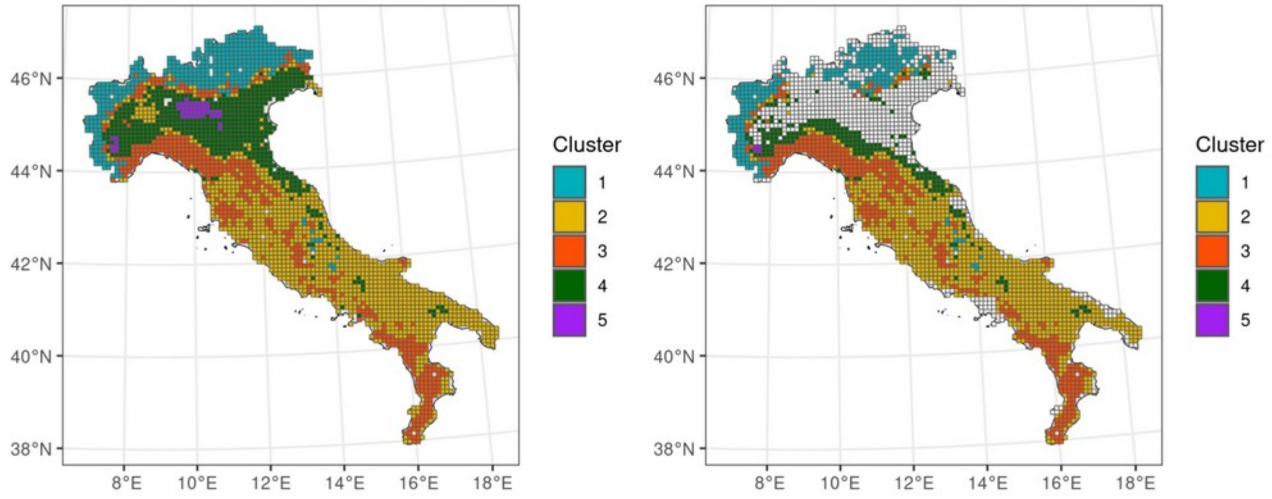

Fig. 2. Spatial distribution of the five clusters obtained through CLARA. Left: distribution of clusters in the Italian peninsula. Right: distribution of clusters in the maximum distribution range of wolves in the Italian peninsula. White cells represent areas outside of the range or with missing data, for which cluster analysis was not possible.

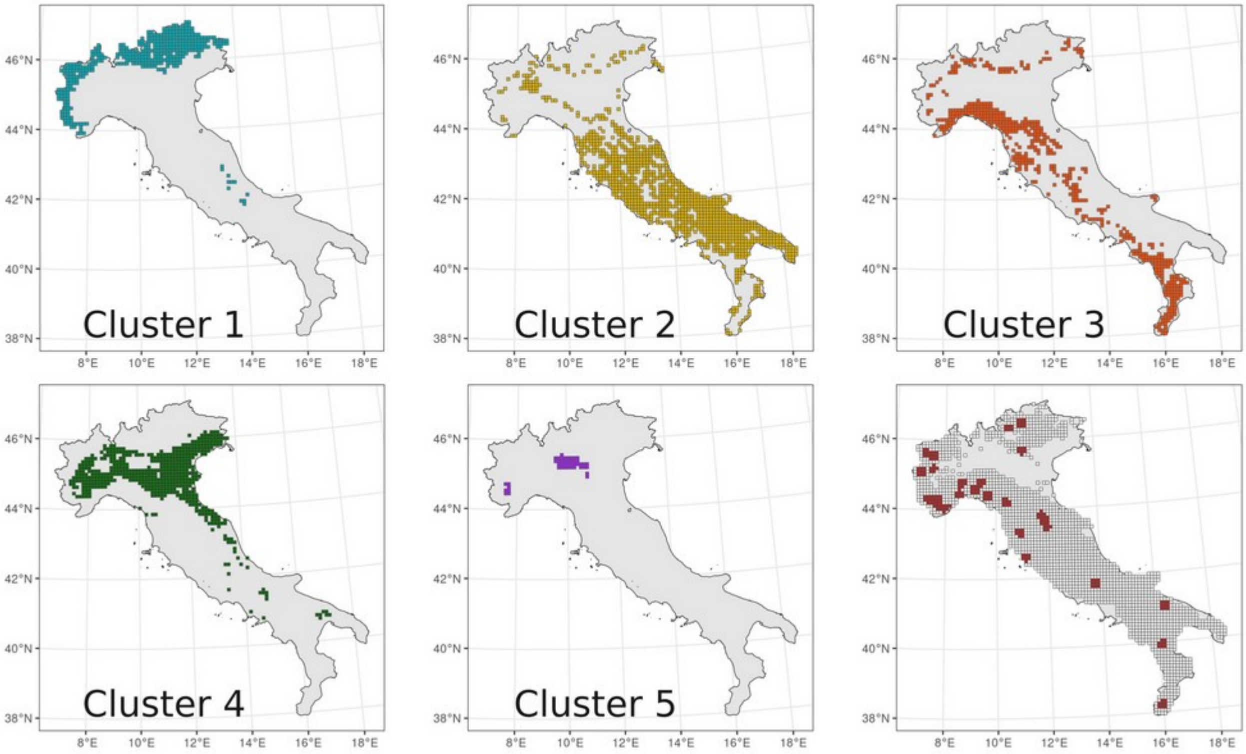

Fig. 3. Spatial distribution in Italy of the five clusters. In the lower-right corner of the figure we also represented the spatial distribution of cells interested by dietary studies (in dark) overlayed on the maximum distribution range of the wolf.

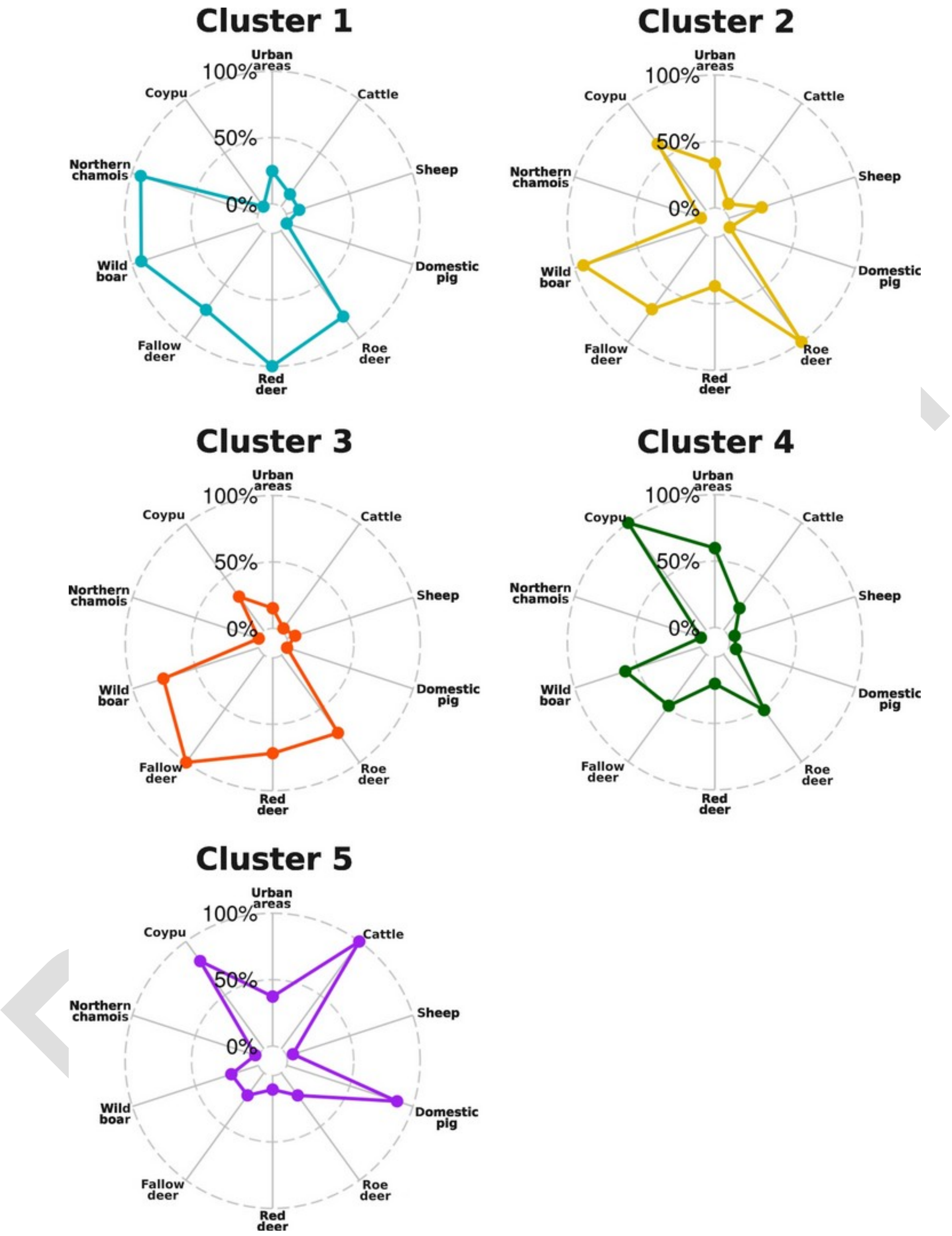

Fig. 4. Median values of environmental covariates in each cluster. Values have been rescaled from 0 to 100, to better interpret them.

Tables

Table 1. Overview of main systematic reviews and meta-analyses about the diet of large carnivores.

Species	**Systematic reviews and meta-analyses about its diet**
African wild dog (*Lycaon pictus*)	Hayward et al., (2006)
American black bear (*Ursus americanus*)	Falconi et al. (2022)
Andean black bear (*Tremarctos ornatus*)	Falconi et al. (2022)
Asiatic black bear (*Ursus thibetanus*)	Falconi et al. (2022)
Brown bear (*Ursus arctos*)	Bojarska and Selva (2012); Falconi et al. (2022); Niedziałkowska et al. (2018)
Polar bear (*Ursus maritimus*)	Falconi et al. (2022)
Brown hyaena (*Parahyaena brunnea*)	-
Cheetah (*Acinonyx jubatus*)	Hayward et al. (2006)
Clouded leopard (*Neofelis nebulosa*)	Chiang and Allen (2017)
Dhole (*Cuon alpinus*)	Srivathsa et al. (2020); Srivathsa et al., (2023)
Dingo (*Canis lupus dingo*)	Fleming et al., (2022); Tatler et al., (2019)
Ethiopian wolf (*Canis simensis*)	-
Eurasian lynx (*Lynx lynx*)	Khorozyan and Heurich (2023a,b)
Gray wolf (*Canis lupus*)	Janeiro-Otero et al., 2020; Meriggi et al., 2011; Meriggi and Lovari, 1996; Mori et al., 2017; Newsome et al., 2016; Zlatanova et al., 2014
Jaguar (*Panthera onca*)	Cruz et al. (2022); Hayward et al. (2016); López-González and Miller (2002); Rubio-Rocha et al. (2023)
Leopard (*Panthera pardus*);	Hayward et al. (2006); Franchini and Guerisoli (2023); Srivathsa et al., (2023); Stein and Hayssen (2013)
Lion (*Panthera leo*)	Hayward and Kerley (2006); Périquet et al. (2014)
Puma (*Puma concolor*)	Cruz et al. (2022); Karandikar et al. (2022); La Barge et al. (2022)
Red wolf (*Canis rufus*)	-
Sloth bear (*Melursus ursinus*)	Falconi et al. (2022)
Snow leopard (*Panthera uncia*)	Lyngdoh et al. (2014); Mallon et al. (2016)
Spotted hyena (*Crocuta crocuta*)	Hayward (2006); Périquet et al. (2014)
Striped hyena (*Hyaena hyaena*)	-
Sun bear (*Helarctos malayanus*)	Falconi et al. (2022)
Sunda clouded leopard (*Neofelis diardi*)	-

Tiger (*Panthera tigris*)	Hayward et al. (2012); Li and Wang (2022); Srivathsa et al., (2023)
Wolverine (*Gulo gulo*)	Fisher et al. (2022)

Table 2. Percentage of cells that were interested by dietary studies, in each one of the three periods (2007-2013, 2014-2018 and 2019-2023), between the five different clusters.

Cluster	Extent, compared to the maximum potential range of the species	Cells covered by studies (overall period)	Cells covered by studies (2007-2013)	Cells covered by studies (2014-2018)	Cells covered by studies (2019-2023)
1	16.1%	28%	34%	21%	32%
2	40.5%	22%	16%	20%	32%
3	20.0%	43%	50%	58%	15%
4	21.6%	8%	0%	2%	22%
5	1.8%	0%	0%	0%	0%